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Mesenchymal stroma cells improve hyperglycemia and insulin deficiency in the diabetic porcine pancreatic microenvironment

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Background

Stem cell differentiation is controlled by extracellular cues from the environment and by intrinsic genetic programs within the stem cell. The present study aimed to explore whether mesenchymal stromal cells (MSC) could improve hyperglycemia and insulin production in the diabetic microenvironment.

Methods

We transplanted male porcine bone marrow-derived EGFP-expressing MSC directly into female diabetic porcine pancreas by multi-point injection. Enzyme-linked immunosorbent assay (ELISA) and fluorescent immunohistochemistry were used to analyze recipients' sera and pancreas tissues for assessment of the therapeutic effect.

Results

Blood glucose levels decreased gradually in MSC-treated recipients from 15 days after the transplantation compared with untreated diabetic controls (15.94± 0.31 mmol/L versus 16.66± 0.11 mmol/L; P = 0.01). Blood insulin increased and glucagons decreased notably in recipients from 2 weeks post-transplantation compared with untreated diabetic controls (0.049± 0.004 μg/L versus 0.037± 0.02 μg/L and 392.9± 20.3 ng/L versus 433.1± 27.6 ng/L). Hematoxylin and eosin (HE)-stained sections demonstrated that the number of islets from each section was markedly increased in recipients compared with that of diabetic controls (10.9± 2.2 versus 4.6± 1.4; P < 0.05) and similar to that of normal controls (10.9± 2.2 versus 12.6± 2.6; P > 0.05). The newly formed islets were smaller than normal islets (47.2± 19.6 μm versus 119.6± 27.7 μm; P < 0.05). Analysis of pancreatic sections for EGFP in recipients indicated that the transplanted MSC survived within the pancreas. Insulin immunoreactivity of pancreatic islets showed that the newly formed islets expressed insulin.

Discussion:

MSC could improve diabetes upon pancreatic microenvironment without obvious immune rejections. This has theoretical and clinical applications.

Keywords

experimental diabetes, marrow stromal cells, pigs, subcapsular pancreas, transplantation.

Introduction

The plasticity of stem cells remains a significant scientific and clinical interest. Regarding optimal treatment of type 1 diabetes, β-cell replacement therapy via stem cell transplantation is a promising possibility. One strategy is to induce stem cells to transdifferentiated into insulin-producing cells *in vitro*, including specific growth factor and analogic pancreatic microenvironment *in vitro* [1–6]. However, an ideal scheme has not been achieved up to now. The transdifferentiated islets do not have the same function as native islets. Another strategy is to transplant diabetic animals with genetically labeled bone marrow (BM) and search for labeled insulin-producing cells in the recipients. However, published reports have presented

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conflicting observations regarding the efficiency of adult stem cell transdifferentiation *in vivo*. Using a CRE-Lox P system, one study revealed that BM harbors cells that have the capacity to differentiate into functionally competent pancreatic endocrine β-cells [7]. A study on mice transplanted with green fluorescent protein (GFP)-positive, sex-mismatched BM did not support the concept that BM contributes significantly to adult pancreatic β-cell renewal [8,9]. The capacity of transplanted BM stem cells to contribute to β-cell regeneration remains controversial [10]. Low transdifferentiation rates and poor function are the main obstacles preventing clinical application. The key point to resolve these two issues is rebuilding a proper microenvironment for stem cell transdifferentiation. However, it is very difficult *in vitro*.

Recent research on stem cells has shown that stem cell function is controlled by intrinsic genetic programs and extracellular cues from the niche that regulates the balance of self-renewal and differentiation. The interaction between stem cells and their supportive microenvironment is critical for their maintenance, function and survival [11–13]. Two studies are discussed. One study indicated that pancreatic extract could stimulate mesenchymal stem cells (MSC) differentiating into insulin-producing cells and increase insulin secretion [2]. Wang *et al.* [14] reconstructed 'personalized' islets by using adult stem cells combined with microfabrication technology. The islets not only showed a normal capacity to produce insulin and glucagons but also less immunoresponse after transplantation. Nevertheless, there are still some issues that need to be resolved: it is difficult to isolate enough islets from the receptor itself [14]. A study on native human (h) MSC has shown that they are able to differentiate into insulin-expressing cells by a mechanism involving several transcription factors of the β-cell developmental pathway when cultured in an appropriate culture conditions [15].

The present study aimed to explore the supposition that the transplanted MSC could decrease glucose and produce insulin upon diabetic microenvironment.

Methods

Animals

Male Chinese Laboratory Miniature pigs (Guizhou Miniature pig strain), aged 8–10 weeks (bought from the Animal Center, Sichuan Province Medical College, China), were used as donors of BM cells. The same strain of 15 female pigs, divided into three groups, normal control (five pigs), diabetic control (STZ + alloxan treated; five pigs) and recipient (Streptozotocin (STZ) + alloxan + MSC; five pigs), was maintained in the animal house of the People's Hospital (Shenzhen, China), on normal chow and water. All procedures were in accordance with the animal experiment guidelines of the Animal Center, Jinan University (Shenzhen, China).

STZ and alloxan-induced experimental diabetic pigs

Female diabetic pigs were induced by three daily auricular vein injections of STZ (freshly prepared in chilled citrate buffer, pH 4.5, 30 mg/kg body weight; Sigma, St Louis, MO, USA) and alloxan (freshly prepared in chilled physiologic saline, pH 7.0, 60 mg/kg body weight; Sigma), as described previously with suitable modifications. Blood glucose levels were monitored once a day using an Accutrend sensor comfort blood glucose meter (Roche Kulmbach GmbH, Kulmbach, Germany). Three days after the last injection, all experimental diabetic pigs became hyperglycemic, with blood glucose levels of 17.3 ± 1.2 mmol/L.

Preparation of EGFP-expressing MSC

Porcine MSC were collected from femurs and tibias of 8–10-week-old donor pigs. The cells were cultured at a density of 1×10^6/mL at 37°C, 5% CO_2. When they reached 80–90% confluence, the cells were digested with trypsin/EDTA (Sigma) for passaging. For phenotypic characterization, MSC markers CD29, CD44, CD45, CD90 and CD106 were measured by flow cytometry (Cytomics FC 500 FACS; Beckman Coulter, Miami, FL, USA), with respective antibodies (Ab) mouse anti-porcine CD45–fluorescein isothiocyanate (FITC), mouse anti-porcine CD44–cy-chrome, mouse anti-porcine CD29–FITC, mouse anti-porcine CD90–phycoerythrin (PE) and mouse anti-porcine CD106–FITC (BD-Pharmingen Co., San Jose, CA, USA).

For EGFP gene transduction, serum-free Opti-MEM (Gibco, Carlsbad, CA, USA) and DNA-lipofectamine2000 flex (1:2.5; Invitrogen, USA) were added to the plate when MSC reached 90–95% confluence. P-IRE-S2-EG vector was constructed in our own laboratory (Department of Immunology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China). Green fluoresce was visualized using inverted fluorescence microscopy (Olympus, London, UK) 48 h after transduction.